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Impact of the host cadaver on survival and infectivity of entomopathogenic nematodes (Rhabditida: *Steinernematidae* and *Heterorhabditidae*) under desiccating conditions

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Abstract

Entomopathogenic nematode species of *Steinernema carpocapsae*, *Steinernema riobrave*, or *Heterorhabditis bacteriophora* were used to compare survival and infectivity among infective juveniles (IJs) emerging in water from hosts in White traps (treatment a), emerging in sand from hosts placed in sand (treatment c), and emerging from hosts placed on a mesh suspended over sand (treatment m). Nematode survival and infectivity was recorded in sand at three-day intervals during 21 days of storage in desiccators at 75% relative humidity and 25 °C. Infectivity was measured by exposing 5 *Galleria mellonella* for 16 h to IJs. Treatment did not affect percent survival of *H. bacteriophora* IJs. Percent survival of *S. riobrave* and *S. carpocapsae* IJs was lowest in treatment a. Across all treatments, by 10 days after the beginning of the experiments, IJ survival declined to 93, 43, and 28% of levels on day 1 for *H. bacteriophora*, *S. riobrave*, and *S. carpocapsae*, respectively. For the three treatments, infection rate over time was described by a negative exponential function for *S. riobrave* and *S. carpocapsae* and by a sigmoid function for *H. bacteriophora*.

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1. Introduction

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* and their associated bacteria *Xenorhabdus* spp. and *Photorhabdus* sp., respectively, are commercially available to control soil insect pests (Georgis and Manweiler, 1994). Entomopathogenic nematode infective juveniles (IJs) search for and infect the insect host (Poinar, 1979). Invasion occurs through natural openings (spiracles, mouth, anus) or, in some cases, directly through the cuticle using a tooth (Kaya and Gaugler, 1993). Once inside the host hemocoele, the nematodes release their associated bacteria, and toxins produced by the nematode and bacteria kill the insect within hours to three days (Burman, 1982). The bacteria provide optimal conditions for nematode reproduction and after 1–3 generations several thousands of new IJs emerge from the insect cadaver. The only free-living stage is a non-feeding third stage juvenile or dauer larva that remains in the soil until it infects a new host or dies (Poinar, 1979).

Once the IJs emerge from the host cadaver they search for a host using different foraging strategies. For example, *Steinernema carpocapsae* remains near the soil surface and uses an ambusher foraging strategy (Campbell and Gaugler, 1993). Others, such as *Heterorhabditis bacteriophora*, move through the soil in search for the host and use a cruiser foraging strategy (Lewis et al., 1992). Still others (e.g., *Steinernema riobrave*) appear to use an intermediate or mixed foraging strategy (from Lewis, 2002).

The dauer larva infectivity (ability to penetrate a host) is often used as a measure of virulence (disease causing power) of entomopathogenic nematodes (Shapiro and Lewis, 1999). Infectivity is also used as an indicator of biological control potential (Glazer, 1991). A problem, however, is infectivity variation among strains, or culture batches of entomopathogenic nematodes in field and laboratory assays (Caroli et al., 1996;

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Lewis and Gaugler, 1994). Also, factors such as the IJs environment after they emerge from the host affect infectivity. Shapiro and Lewis (1999) reported that *H. bacteriophora* IJ infectivity was lower when IJs were applied to sand in a water suspension than when infected cadavers were applied and IJs emerged naturally into sand.

Entomopathogenic nematodes are routinely stored or applied to soil for insect control in aqueous suspension (Woodring and Kaya, 1988). There is no information on IJs long-term survival and infectivity using application methods other than IJs in water suspension. Accordingly, our objective was to compare survival and infectivity among IJs that emerged into sand from the host, crawled into sand from a host suspended on a wire mesh, or were applied to sand in aqueous suspension using three nematode species with different foraging strategies.

2. Materials and methods

We compared survival and infectivity among IJs emerging under three different treatments. Treatments were emergence of IJs into water from hosts in White traps (White, 1927) (treatment a), emergence of IJs directly into sand from hosts placed in sand (treatment c), and emergence of IJs from hosts placed on a mesh suspended over sand (treatment m). Entomopathogenic nematode species tested were *S. carpocapsae* (Weiser) (All strain), *S. riobrave* Cabanillas, Poinar and Raulston (Texas strain), and *H. bacteriophora* Poinar (Hb strain). All experiments were conducted at ca. 25 °C and at 75% relative humidity. Nematodes used to initiate experiments were cultured in the last instar of the greater wax moth, *Galleria mellonella*, according to procedures described by Woodring and Kaya (1988).

Wax moth larvae were exposed individually to IJs (<15 days old) in 24-well (1.5-cm diam.) plates and lined with two filter paper discs (Whatman no. 1). Exposure rates were approx. 50, 50, or 100 IJs of *S. riobrave*, *S. carpocapsae*, and *H. bacteriophora*, respectively, per wax moth larva. Three days after inoculation, the infected wax moth larvae were divided among the three treatments. Infective juveniles collected from 12 infected wax moth larva cadavers were used for each treatment. For the aqueous treatment (a), nematode-infected wax moth larvae were placed individually in White traps for later collection of IJs from water. For the cadaver treatment (c), 12 nematode-infected wax moth larvae were placed over sand (10% water w/w) contained in a petri dish (150 mm). For the mesh treatment (m), 12 nematode-infected wax moth larvae were placed on a metallic mesh mounted over sand (10% water w/w) contained in a petri dish (150 mm).

Wax moth larva cadavers from the three treatments where checked daily for IJ emergence. Three days after the beginning of IJ emergence, nematodes from the White traps (treatment a) were concentrated with a vacuum pump onto nitrocellulose paper with 0.3 μm openings (Millipore, Bedford, MA). The concentrated nematodes were collected with a spatula from the nitrocellulose paper and applied to a petri dish (150 mm) containing sand with 10% water (w/w). Also, three days after IJ emergence, individual wax moth larvae were removed from the cadaver and mesh treatments. Consequently, the number of IJs applied to each petri dish filled with sand was the total of the first three days of emergence of 12 wax moth larva cadavers.

Three experiments were conducted separately in desiccators maintained at 75% relative humidity with a NaCl-saturated salt solution (Winston and Bates, 1960). In each experiment, an experimental unit consisted of a petri dish (150 mm) filled with moist sand (10% water w/w) that contained IJs (from treatment a, c, or m) of one of the three entomopathogenic nematode species. Each treatment was replicated four times and each experiment was conducted twice.

Four days after IJ emergence from the host cadaver (considered as day 1 in the statistical analysis) and every three days thereafter, one 10-cm^3-sand sample was taken from each experimental unit and infectivity and survival of the IJs therein was recorded. Infectivity was tested by exposing five wax moth larvae for 16 h to the approx. 10-cm^3-sand sample in a petri dish (60 mm). The petri dishes were sealed with parafilm and kept in an incubator at 25 °C. After the exposure period, the wax moth larva cadavers were removed from the sand sample, rinsed and left for 48 h at ambient temperature before storage at −20 °C. Wax moth cadavers were dissected using the pepsin digest method (Caroli et al., 1996; Mauleon et al., 1993) and the number of infecting nematodes was recorded. After removal of wax moth larva cadavers from the sand, IJs were extracted from the sand by sedimentation in tap water. Live and dead IJs were counted. Nematodes were considered live if they were naturally moving or responded to probing with a fine needle. Percentage of infecting nematodes (number infecting/number infecting + number alive + number dead) and percentage of live nematodes (number alive + number infecting/number alive + number infecting + number dead) were calculated for each sample and sampling date.

The experimental design was a split-plot design. Whole plot factors were the three treatments (aqueous, cadaver, and mesh) arranged in a completely randomized design for *S. carpocapsae* and *S. riobrave* and in a completely randomized block design for *H. bacteriophora*. Sub-plots were the five sampling dates (samples were taken the first day dishes were placed in the desiccators and every three days thereafter until day 21). Data were subjected to analysis of variance for a split-plot design (Montgomery, 1991) using SAS (SAS Institute, Cary, NC). Mean percentages of live or infecting IJs were compared within dates among treatments using Duncan's multiple-range test for each entomopathogenic nematode species.

Mean percentage of live and infecting nematodes (y) were transformed to $y = \ln(y + 1)$. For each entomopathogenic nematode species within treatments, y and $\ln(y + 1)$ were fitted to sampling dates (x) using least squares analysis. Models with the best fit (highest r^2) are presented.

3. Results

Analysis showed no interaction between the two tests run for each of the three nematode species. Therefore, results from duplicate tests were combined for final analysis. Nematode survival differed on several sampling dates among treatments ($P \leqslant 0.05$) for *S. carpocapsae* and *S. riobrave* IJs but not for *H. bacteriophora* IJs (Table 1). Across all sampling dates, *S. carpocapsae* survival in treatment a (10.3%) was less ($P \leqslant 0.05$) than survival on treatments c (16.7%) or m (17.0%). Also for *S. riobrave* across all sampling dates nematode survival in treatment a (13.6%) was less ($P \leqslant 0.05$) than survival in treatments c (34.8%) or m (35.7%) (Table 1). Nematode infection rate differed among treatments on four sampling dates for *S. carpocapsae*, on two sampling dates for *S. riobrave*, and one sampling date for *H. bacteriophora* (Table 1).

For *S. carpocapsae*, the relationship between log of percent survival ($\log y$) and days after emerging from wax moth larva cadavers (x) was linear for all treatments (Fig. 1). Treatment a, $r^2 = 0.86$, $P \leqslant 0.05$ (Fig. 1A); treatment c, $r^2 = 0.84$, $P \leqslant 0.05$ (Fig. 1B); treatment m, $r^2 = 0.96$, $P \leqslant 0.05$ (Fig. 1C). The regression slopes (rate of decrease of live nematodes) were not significantly different among the three treatments ($P \geqslant 0.05$) (Fig. 1). For *S. riobrave*, the relationship between log of percent survival ($\log y$) and days after emerging from wax moth larva cadavers (x) was linear for all treatments (Fig. 2). Treatment a, $r^2 = 0.96$, $P \leqslant 0.05$ (Fig. 2A); treatment c, $r^2 = 0.84$,

Table 1
Survival (%) and infection (%) of *S. carpocapsae*, *S. riobrave*, and *H. bacteriophora* at different times in desiccator at 75% relative humidity

DAE[a]	Survival (%)			Infection (%)		
	a	c	m	a	c	m
S. carpocapsae						
1	36a[b]	41a	51a	22b	13ab	9a
5	13a	16a	27a	11a	8a	3a
9	3a	17b	10ab	3.1a	2a	7b
13	7a	18b	5a	0.5a	2b	0.5a
17	2a	6b	2a	0.5a	2b	0.5a
21	0.5a	2a	7b	0.1a	0.1a	0.3a
Mean	10.3a	16.7b	17.0b	6.2a	4.5a	3.4a
S. riobrave						
1	37a	61b	64b	12a	14a	26a
5	21a	50b	53b	3a	5b	7b
9	14a	43b	45b	1a	7b	4ab
13	5a	28b	27b	1a	3a	5a
17	4a	25a	16ab	1a	0.3a	2a
21	0.5a	2a	9b	0.3a	0.7a	0.2a
Mean	13.6a	34.8b	35.7b	3.1a	5.0ab	7.4b
H. bacteriophora						
1	96a	87a	97a	1.6a	1.3a	1.1a
5	92a	82a	94a	5a	5a	7a
9	93a	86a	97a	14a	15a	12a
13	72a	68a	79a	15a	16a	15a
17	49a	70a	56a	10a	16b	15a
21	23a	22a	18a	7a	4a	5a
Mean	70.8a	69.2a	73.5a	8.8a	9.6a	9.2a

Treatment a, infective juveniles (IJs) applied to sand in water suspension after collection from a White trap; treatment c, IJs emerging from cadavers into sand; treatment m, IJs that crawled into sand from a suspended mesh.

[a] Days after emergence from *G. mellonella*.

[b] Data are combined means of two tests each with four replicates. For survival (%) and infection (%) within a row, data followed by the same letter are not significantly different according to Duncan's multiple range test.

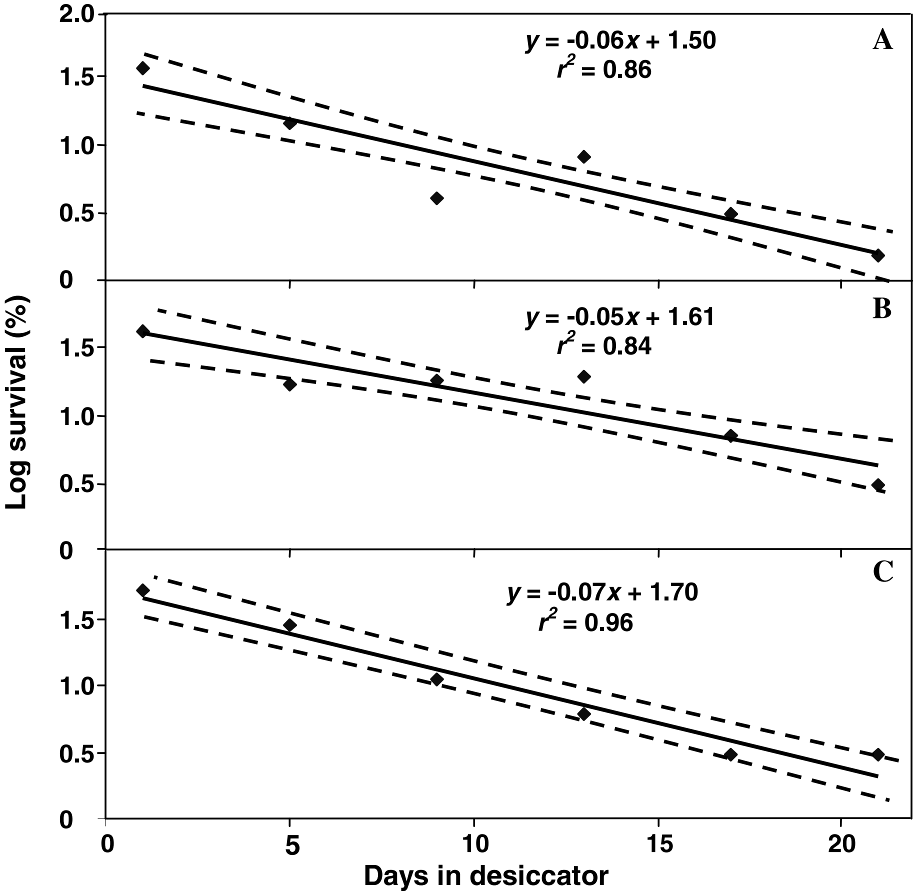

Fig. 1. Logarithm of survival (%) of *S. carpocapsae* infective juveniles (IJs) over time at 75% relative humidity for different treatments. Infective juveniles applied to sand in water suspension after collection from a White trap (A), IJs emerging from cadavers into sand (B), IJs that crawled into sand from a suspended mesh (C). Dotted line represents upper and lower 95% confidence limits.

$P \leqslant 0.05$ (Fig. 2B); treatment m, $r^2 = 0.95$, $P \leqslant 0.05$ (Fig. 2C). The regression slope of treatment a was significantly different (faster drop in survival) from the slopes of treatments c or m ($P \leqslant 0.05$) (Fig. 2). For *H. bacteriophora*, the relationship between percent survival (y) and days after emerging from wax moth larva cadavers (x) was quadratic for all treatments (Fig. 3). Treatment a, $r^2 = 0.98$, $P \leqslant 0.05$ (Fig. 3A); treatment c, $r^2 = 0.88$, $P \leqslant 0.05$ (Fig. 3B); treatment m, $r^2 = 0.99$, $P \leqslant 0.05$ (Fig. 3C).

For *S. carpocapsae* and *S. riobrave*, the relationship between percent of infecting IJs (y) and days after emerging from wax moth larva cadavers (x) was described by a negative exponential function of the form $y = a\mathrm{e}^{-bx}$ (*S. carpocapsae*: treatment a, $r^2 = 0.97$, $P \leqslant 0.05$; treatment c, $r^2 = 0.81$, $P \leqslant 0.05$; treatment m, $r^2 = 0.81$, $P \leqslant 0.05$; *S. riobrave*: treatment a, $r^2 = 0.84$, $P \leqslant 0.05$; treatment c, $r^2 = 0.79$, $P \leqslant 0.05$; treatment m, $r^2 = 0.81$, $P \leqslant 0.05$) (Figs. 4A and B). For *H. bacteriophora*, the relationship between percent of infecting IJs (y) and days after emerging from wax moth larva cadavers (x) was described by polynomial function of the form: $y = -ax^3 + bx^2 + bx$ (treatment a, $r^2 = 0.99$, $P \leqslant 0.05$; treatment c, $r^2 = 0.87$, $P \leqslant 0.05$; treatment m, $r^2 = 0.99$, $P \leqslant 0.05$) (Fig. 4C). From the formula, $y = -ax^3 + bx^2 + bx$, the day (value of x) of maximum infection rate for each treatment was calculated by finding the value of x when the first derivative $y' = 0$. The highest infection rate by *H. bacteriophora* occurred on day 14 for treatment c (16%), day 13 for treatment m (13%), and day 10 for treatment a (10%) (Fig. 4C).

4. Discussion

We presented evidence that water suspension of IJs after emergence from the host cadaver can affect survival and infectivity in laboratory experiments. Generally, when the effect of suspending IJs in water was significant it lowered survival and infectivity on the three nematode species tested. Models describing survival of IJs over time were quadratic for *H. bacteriophora* and linear for the two *Steinernema* species. Across

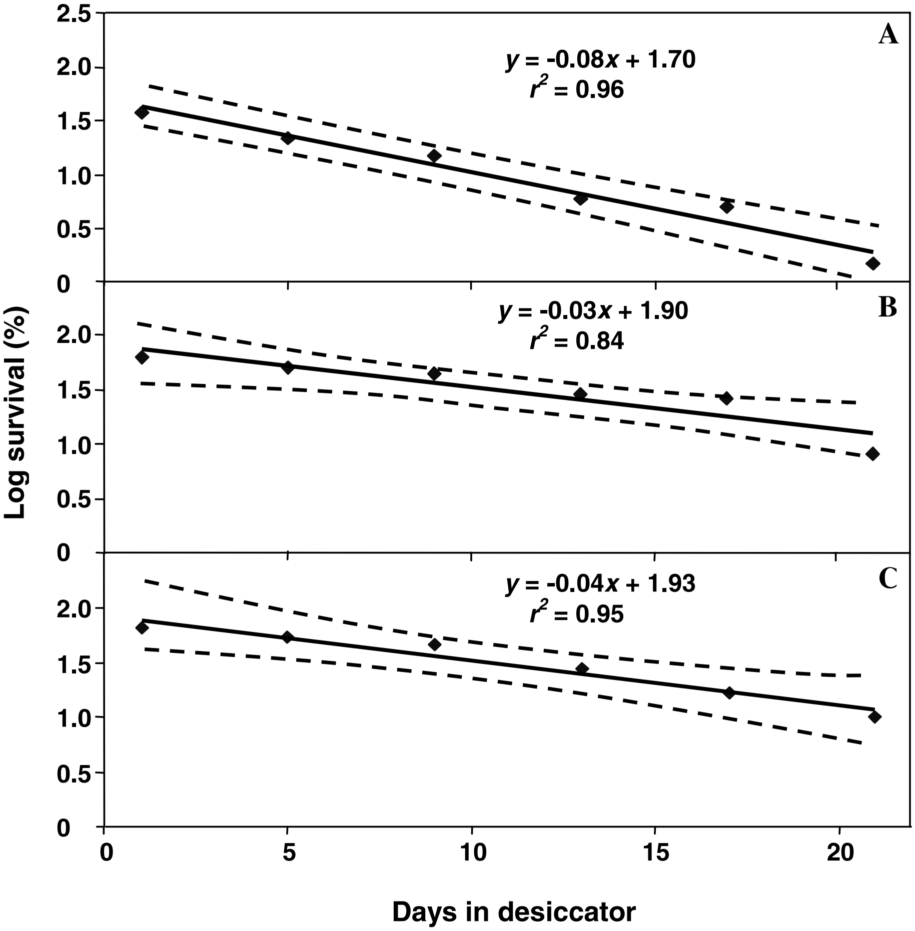

Fig. 2. Logarithm of survival (%) of *S. riobrave* infective juveniles (IJs) over time at 75% relative humidity for different treatments. Infective juveniles applied to sand in water suspension after collection from a White trap (A), IJs emerging from cadavers into sand (B), IJs that crawled into sand from a suspended mesh (C). Dotted line represents upper and lower 95% confidence limits.

all treatments by 10 days after the beginning of the experiments, IJs declined to 93, 43, and 28% of levels on day 1 for *H. bacteriophora*, *S. riobrave*, and *S. carpocapsae*, respectively. The lowest mortality of *H. bacteriophora* early in the experiment may have been due to its cruiser foraging strategy (Lewis et al., 1992). The moist sand arena may have buffered desiccation of *H. bacteriophora* IJs early in the experiment and could explain the quadratic model of survival over time. *S. carpocapsae*, an ambusher and surface dweller, may have not been buffered by the sand arena and had the highest mortality early in the experiment.

Survival rates of the three nematode species in our experiments were higher than those reported previously in field experiments (Duncan et al., 1996; Duncan and McCoy, 1996). Possibly the moisture depletion rate was slower under our laboratory conditions than under the field conditions in previous experiments. A slower moisture depletion rate may allow IJs to adapt to a desiccating environment by entering a partially anhydrobiotic state (Womersley, 1990). The use of sterilised sand in our experiments may have also increased nematode survival rate. Nematodes were reported to survive more time in sterilised than in unsterilised soil due to a lack of nematode antagonists in sterilised soil (Hass et al., 2001; Timper et al., 1991).

Percentage of infecting *H. bacteriophora* IJs over time had a sigmoid shape with maximum at days 10, 13, and 14 for treatments a, m, and c, respectively. For *H. bacteriophora*, in only one sampling date was the percent of infecting IJs in the c treatment significantly higher than those of the two other treatments. Shapiro and Lewis (1999) found greater *H. bacteriophora* infectivity when IJs emerged directly from the cadaver compared to IJs applied in aqueous suspension. Based on this previous report we expected a greater treatment effect on *H. bacteriophora* infectivity. However, Shapiro and Lewis (1999) recorded percent of infecting IJs after 24- and 48-h exposure periods of 10 wax moth larvae

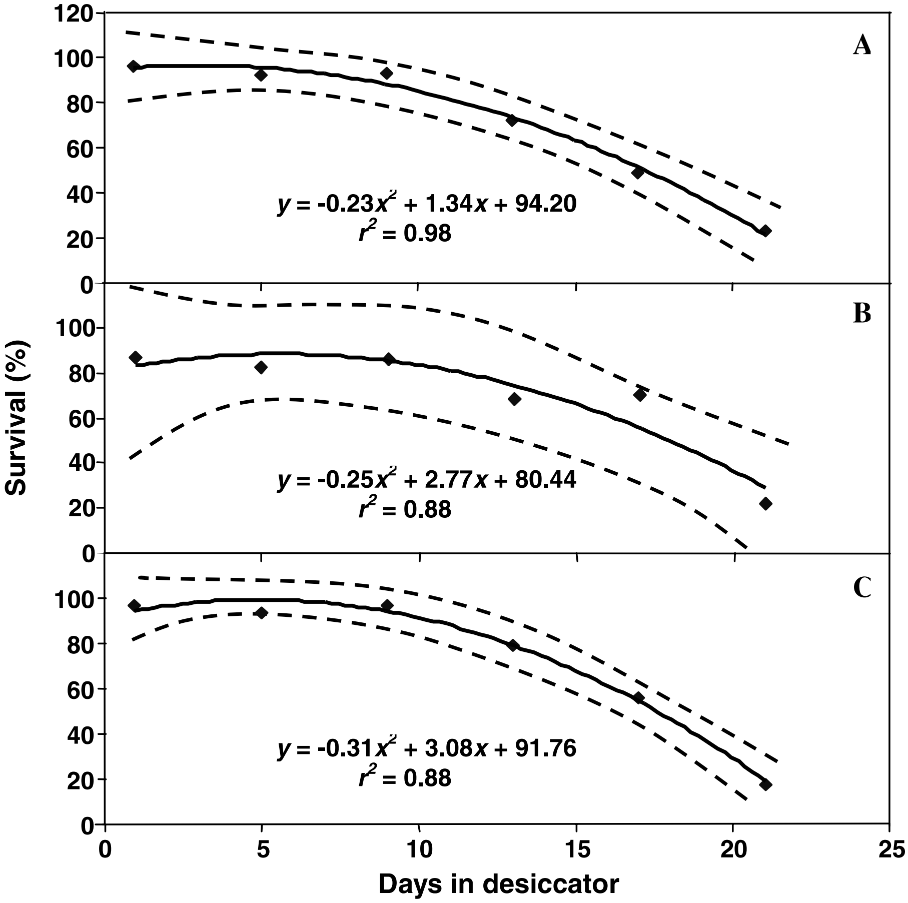

Fig. 3. Survival (%) of *H. bacteriophora* infective juveniles (IJs) over time at 75% relative humidity for different treatments. Infective juveniles applied to sand in water suspension after collection from a White trap (A), IJs emerging from cadavers into sand (B), IJs that crawled into sand from a suspended mesh (C). Dotted line represents upper and lower 95% confidence limits.

whereas we exposed five wax moth larvae to IJs for a 16-h exposure period. Host exposure time to IJs has been positively correlated with infection rate (Epsky and Capinera, 1993). Increased exposure time of wax moth larvae to IJs (e.g., 24 and 48 h) may have produced results similar to those of Shapiro and Lewis (1999).

The fluctuation (i.e., increase) in *H. bacteriophora* IJs infection rate over time is consistent with the hypothesis that a large proportion of the entomopathogenic IJ population is temporarily non-infectious (Bohan and Hominick, 1995). Similar to our data, other studies reported infectivity increases of *Heterorhabditis* spp. IJs after storage (Griffin, 1996; Griffin and Downes, 1994). A gradual IJ maturation process or diapause may be responsible for an increase of heterorhabditid infectivity with time (Griffin, 1996; Womersley, 1993). Infectivity patterns between *H. bacteriophora* and the two *Steinernema* species were different in our experiments. This finding supports a previous report that a phased infectivity pattern may hold for heterorhabditids but not for steinernematids (Campbell et al., 1999).

This study is one of several that examines the effects of water storage and collection of entompathogenic nematode IJs on their behavior, ecology and biological control potential. Infective juveniles that emerged directly from the cadavers had greater dispersal and infectivity rates than IJs applied in aqueous suspension (Shapiro and Glazer, 1996; Shapiro and Lewis, 1999). Water suspension of IJs after emergence from the insect host may cause osmotic stress and affect their fitness. Host insect cadavers may function as a buffer to extreme environmental conditions enabling IJs to persist longer (Koppenhöfer et al., 1995). Furthermore, efficacy of the "applied cadaver approach" was demonstrated in field experiments using *Heterorhabditis* spp. and high levels of *Heterorhabditis* spp. IJs persisted in the field 230 days after application (Jansson et al., 1993). Further research will increase the understanding of the cadaver's role in entomopathogenic nematode ecology.

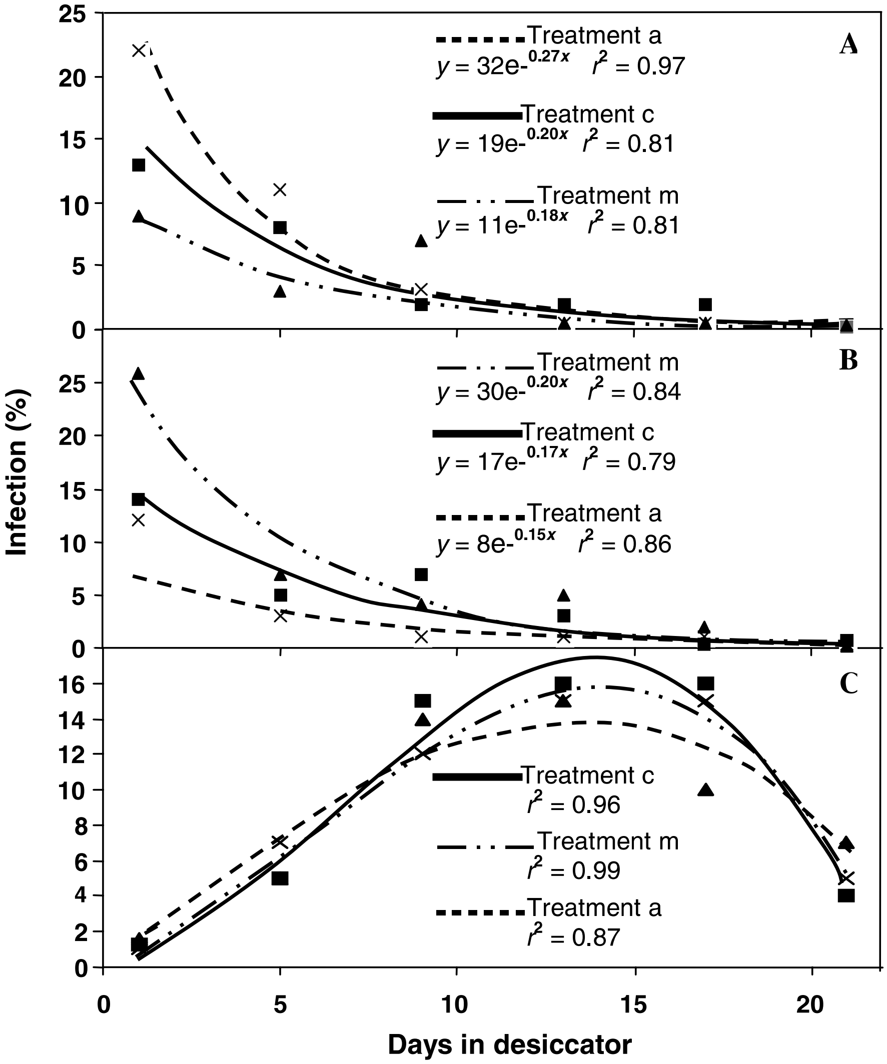

Fig. 4. Percent of infective juveniles (IJs) parasitizing five *G. mellonella* after a 16-h exposure period to IJs stored in sand (10% water) for various times in desiccator at 75% relative humidity. *S. carpocapsae* (A), *S. riobrave* (B), and *H. bacteriophora* (C). Treatment a, IJs applied to sand in water suspension after collection from a White trap; treatment c, IJs emerging from cadavers into sand; treatment m, IJs that crawled into sand from cadavers placed on a mesh suspended over sand.

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